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Organization and evolution chalcone synthase gene family in bread wheat and related species

A.Yu. Glagoleva[1, 2]*, E.K. Khlestkina[1, 2]
[1] *Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia*
[2] *Novosibirsk State University, Novosibirsk, Russia*
** e-mail: glagoleva@bionet.nsc.ru*

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Motivation and Aim: Flavonoids are secondary plants metabolites, the most of these compounds are related with coloration traits in plants, which makes them a convenient model for genetics studies. Chalcone synthase (CHS) is a key enzyme of the flavonoid biosynthesis pathway and is involved in the biosynthesis of all classes of flavonoid compounds. The genes encoding this enzyme are usually represented in the plants genomes in many copies and in most species are described in detail. Nevertheless, in bread wheat (*Triticum aestivum* L.) these genes have not been explored yet. The purpose of this study was to investigate the structural and functional organization of the chalcone synthase gene family, and its evolution in bread wheat and relative species.
Methods and Algorithms: Homologous sequences search was performed using BLAST algorithm in two databases (https://urgi.versailles.inra.fr/blast/blast.php, www.ncbi.nlm.nih.gov/Database/) within genomic sequences of *T. aestivum* and its tetraploid (*T. durum*) and diploid (*T. monococcum*, *T. urartu, Aegilops speltoides*, *Ae. sharonensis*, and *Ae. tauschii)* relatives. Multiple sequence alignment was done with MULTALIN 5.4.1. Cluster analysis was performed with MEGA v6.06 software using Neighbor-Joining algorithm. To design a set of copy-specific primer pairs PrimerQuest Tool (https://eu.idtdna.com/Primerquest/Home/Index) was used. These primers were exploited for PCR from DNA of nulli-tetrasomic and deletion lines and RT-PCR from cDNA for pericarp, coleoptile and root.
Results: The nucleotide sequences of the five *Chs* copies in *T. aestivum* were identified. Among them three homeologous gene copies in A- and D-genomes (*Chs-A1*, *Chs-B1* and *Chs-D1*) and two paralogous gene copies in B-genome (*Chs-B2*, *-B3*). It was shown that all *Chs* gene copies are located on the distal regions of 2AS, 2BS and 2DS chromosomes. All copies with the exception of *Chs-B2* transcribed in colored pericarp and coleoptile, but they were not transcriptionally active in colorless pericarp and root. *Chs-B2* was transcribed in colored coleoptile only. Analysis of transcriptional activity and the structure of the promoters of copies of the *Chs* copies showed that they participate in the synthesis of different classes of flavonoid compounds in different (both optimal and stressful) conditions. To clarify the origin of paralogous *Chs* duplications in the B genome, we compared sequences of *Chs* genes in different *Triticum* and *Aegilops* species and calculated the time of divergence of the paralogues.
Conclusion: First *Chs* duplication event took place in common ancestor of *Triticum* and *Aegilops* about 10 MYA, then one of the copies was again duplicated 6-7 MYA in the ancestor of the B-genome, while other copy likely pseudogenized in *T. aestivum* 2A and 2D chromosomes. The specialization of individual copies seems to be the reason for maintaining five *Chs* gene copies in the genome of *T. aestivum*.
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